Neuron, Vol. 23, 645–648, August, 1999, Copyright ©1999 by Cell Press

mRNA Trafficking and Local Protein Synthesis at the Synapse

Minireview

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Most cells are heterogeneous entities in which specific regions are specialized for particular functions, as reflected by the uneven distribution of intracellular organelles. Neurons are no exception. Besides the intrinsic polarity used to separate input–output functions (dendrite versus axon), there exist further functional specializations within each of these polarized domains. For example, the release probabilities may differ between the boutons of a given axon. In addition, the spines within a single dendritic tree exhibit substantial diversity in shape, protein composition, and ability to respond to neurotransmitter. This uniqueness of individual boutons or spines is established and maintained by differences in the types and concentrations of proteins present, as well as their modification state. The specific targeting of proteins synthesized in the soma no doubt contributes to this heterogeneity of synaptic boutons and spines (see accompanying minireview by Foletti et al., 1999 [this issue of *Neuron*]). An additional mechanism for establishing and maintaining this diversity involves the localized transport and translation of mRNAs within dendrites or axons. This minireview focuses on recent findings that shed light on the mechanisms of dendritic mRNA transport and protein synthesis, with an emphasis on how such processes might contribute to synaptic plasticity.

Dendritic mRNA Targeting and Localization

The control of mRNA localization is an important general mechanism used to restrict proteins to particular domains of many polarized cells, including neurons. Localizing mRNAs may permit cells to achieve high levels of protein expression at the site of localization, thus facilitating a quick supply of particular proteins. In addition, together with protein translation control mechanisms, mRNA targeting may allow for the exclusion of proteins from particular cellular domains. The best-studied example of mRNA localization involves the segregation of specific messages in the egg of developing *Drosophila*. In *Drosophila* oocytes, *bicoid*, *prospero*, and *oskar* mRNAs are localized to specific subdomains of the egg, thus directing cell development and anterior–posterior patterning (reviewed by St. Johnston, 1995; Bashirullah et al., 1998). Several mRNAs, such as *MAP2*, activity-related cytoskeletal protein (*ARC*), fragile X mental retardation protein, and the α subunit of *CAMKII*, have also been shown to be localized to neuronal dendrites (reviewed by Steward, 1997). For many localized mRNAs, *cis*-acting sequences required for localization are found in the 3′ untranslated region (3′ UTR) of each transcript (St. Johnston, 1995). This is also true for at least two of the transcripts that have been found in neurons, the α subunit of *CAMKII* mRNA and the β-*actin* mRNA. Primary sequence analysis of the 3′ UTR reveals little or no homology among identified dendritically localized messages. How, then, do these *cis*-acting regions in the 3′ UTR confer localization? One possibility is that RNA-binding proteins recognize specific secondary structures in the 3′ UTR, forming complexes that are then transported along microtubules. For example, in *Drosophila*, the double-stranded RNA-binding protein Staufen associates with *bicoid*, *oskar*, and *prospero* mRNA and is required for their localization (Bashirullah et al., 1998; see below).

Relatively little is known about the dynamics of mRNA transport in dendrites. Studies using cell-permeant nucleic acid dyes (SYTO dyes) have permitted observations of RNA-containing granules moving along microtubules in dendrites (Knowles et al., 1996). The visualized RNA particles colocalized with poly(A) mRNA, the 60S ribosomal subunit, as well as elongation factor 1α, suggesting that the granules may represent translational units or complexes (Knowles et al., 1996). How individual mRNAs get recruited to these complexes remains unknown, although a likely mechanism involves recognition of mRNAs by *trans*-acting RNA-binding proteins. In *Drosophila*, the Staufen protein associates with similar RNA-containing and microtubule-associated granules (St. Johnson, 1995). In mammals, several homologs of Staufen have recently been identified (Kiebler et al., 1999; Wickham et al., 1999). Immunohistochemical localization of a rat Staufen homolog revealed a somatodendritic localization in cultured hippocampal neurons and an association with RNA-containing granules, rough endoplasmic reticulum, and microtubules (Kiebler et al., 1999). In vitro binding assays indicated that mouse Staufen binds all double-stranded RNAs tested (Wickham et al., 1999), similar to what has been observed for *Drosophila* Staufen (St. Johnston, 1995). It is unclear whether this lack of specificity will hold true when binding partners are assessed in vivo, as the availability of Staufen, interacting proteins, and potential inhibitors of binding may well influence the specificity. Although Staufen's association with mRNAs is apparent in vitro, it is still not known whether Staufen is actually responsible for recruiting mRNAs to the complex and then directing mRNAs to the dendrites. Overexpression, dominant-negative, and knockout strategies will no doubt soon address these issues.

Also poorly understood is how individual mRNA-containing complexes are anchored to the appropriate dendritic sites. It is likely that the docking of mRNA complexes involves a shift from a microtubule-based transport to a cytoskeletal-based anchor, as has been shown in nonneuronal systems (reviewed by Bashirullah et al., 1998). A neuronal mRNA-anchoring mechanism has two potential components. The first involves the general recognition of a translation site within the dendrite (usually observed adjacent to synaptic sites). This

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could be accomplished by the interaction of synapse-associated cytoskeletal proteins or proteins of existing polyribosomal complexes with proteins resident in the mRNA complex. The second involves the recognition of particular mRNAs within a complex and the subsequent sequestration or rejection of that complex. This mechanism is needed if there happens to be heterogeneity in the mRNA composition of different complexes. Whether different mRNA complexes contain different complements of mRNAs is an open question, but some data seem to support this view (see below). The molecular markers for these putative heterogeneous mRNA complexes could be associated proteins or the mRNAs themselves. According to this idea, one could envision synaptic events modifying local proteins that would then recognize markers on the specific mRNA complexes, leading to translation of these specific mRNAs and, potentially, modulation of synaptic function.

If dendritic protein synthesis is important for establishing and maintaining the uniqueness of individual synapses, it follows that protein translation (see below) and/or delivery of mRNAs to dendritic compartments might be regulated. In the latter case, there is evidence for at least two kinds of regulation: the first influences overall mRNA flow to the entire dendritic domain; the second determines which specific subset of synapses will receive or stabilize newly transported messages. Pharmacological manipulations suggest that altering synaptic activity can modulate the bulk flow of mRNA to the dendrites. For example, treatment of cultured hippocampal neurons with an elevated K^+ solution can result in the detection of both brain-derived neurotrophic factor (*BDNF*) and *TRKB* mRNA in the distal regions of the dendrites (Tongiorgi et al., 1997). In addition, treatment of SYTO-labeled cortical cultures with the growth factor neurotrophin-3 resulted in an increase in the translocation of RNA granules to distal processes (Knowles and Kosik, 1997). Other experiments suggest that the availability of individual mRNAs can be fine-tuned to specific dendritic regions. For example, *MAP2* mRNA is usually localized in the proximal dendrite, whereas the mRNA for *CAMKII*α is distributed throughout the dendrite. A PCR-based study of mRNA identities in different branches of the same cultured neurite revealed substantial heterogeneity among the mRNAs found in neighboring branchlets (see Steward, 1997). In addition, synaptic activity can regulate *ARC* mRNA appearance in hippocampal granule cell dendrites, as detected by in situ hybridization (Steward et al., 1998). In these experiments, the *ARC* mRNA was restricted to a previously activated dendritic area, suggesting that targeting mechanisms exist to restrict *ARC* message delivery to appropriate sites. Alternatively, the *ARC* mRNA could be globally distributed and selectively stabilized at the previously activated dendritic site. In order to address this issue, the recent development of techniques that allow the dynamic visualization of specific mRNA species may prove useful (Bertrand et al., 1998).

Protein Translation in the Dendrite

Early anatomical studies demonstrated that polyribosomes are localized beneath or within spines (reviewed by Steward, 1997). These observations, coupled with demonstrations of distinct mRNA species localized in dendrites, motivated experiments exploring whether protein synthesis can occur in dendrites. Biochemical and immunohistochemical experiments suggest that dendrites contain glycosylating activity as well as some rough endoplasmic reticulum and Golgi apparatus proteins (reviewed by Steward, 1997). Several groups have now observed new protein synthesis in biochemical fractions containing isolated synaptic fragments in transected neurites of cultured neurons and in brain slices (reviewed by Schuman, 1997; Steward, 1997). These experiments clearly indicate that dendrites possess the capability to synthesize new proteins—the challenge remaining is to understand how local translation is regulated by synaptic activity and whether or not it contributes to synaptic plasticity.

What intracellular signaling molecules might couple synaptic activity to the translation machinery? In many cells, growth factors activate a rapamycin−sensitive signaling pathway, resulting in the translation of a subset of eukaryotic mRNAs. Important components of this pathway include the cap−binding protein elongation initiation factor (EIF) eIF-4E, eIF-4E binding proteins (4E−BPs), and a kinase known as mammalian-target-of-rapamycin (mTOR, a.k.a. RAFT1 or FRAP; reviewed by Brown and Schreiber, 1996). A recent study has identified the synaptic protein gephyrin as a MTOR-binding protein (Sabatini et al., 1999). The association of MTOR with gephyrin is required for rapamycin-sensitive protein translation in HEK 293 cells (Sabatini et al., 1999). Gephyrin was originally identified as a protein that interacts with glycine receptors and is required for their clustering in spinal cord neurons. In situ hybridization analysis has shown that gephyrin is also present in brain, suggesting a potentially broader function for gephyrin in the CNS. In another study in tadpole tecta, the application of NMDA or visual stimulation results in the phosphorylation of eukaryotic translation elongation factor 2 (eEF2), resulting in the cessation of ribosomal translocation (Scheetz et al., 1998).

Translational repression may also serve an important regulatory role in dendritic protein synthesis. In *Drosophila* embyrogenesis, proteins such as Bicoid, Pumilio, and Smaug bind to sequences in the 3′ UTR of specific mRNAs and prevent their translation (Bashirullah et al., 1998). Translational repression that is spatially restricted to one end of the *Drosophila* embryo is achieved by the uneven distribution of the repressor protein. In neurons, the presence of repressor proteins could prevent translation of mRNAs en route from the soma to dendritic sites. In addition, repressor proteins might also serve to prevent mRNA translation at particular sites. Synaptic activity could result in the relief from repression or, alternatively, repress the translation of particular mRNAs that limit or oppose the plasticity of the synapse. As of yet, no putative repressors of dendritic mRNA translation have been identified.

An additional mechanism for the control of translation of a specific mRNA is the modulation of the length of the poly(A) tail at the 3′ end of the mRNA. Increases in poly(A) length are usually associated with increases in protein translation, whereas decreases in poly(A) length are associated with repression. For example, the translational repression mediated by proteins such as pumilio (see above) is accomplished by rapid deadenylation of the target mRNA. Increases in poly(A) tail length in the

cytoplasm are promoted by the presence of a cytoplasmic polyadenylation element (CPE) in the 3′ UTR of some mRNAs. The *CAMKIIα* mRNA contains two such CPE sequences. It has recently been shown that visual experience can cause a rapid polyadenylation and translation of *CAMKII* mRNA in the visual cortex of dark-reared rats (Wu et al., 1998). Although it was not directly demonstrated that CAMKII synthesis occurred in the dendrites, the data suggest this possibility. Coupled with the demonstrations of dendritic protein synthesis in vitro, described below, these observations suggest the capacity for local control of synaptic transmission.

Local Synthesis during Plasticity

What is the evidence that locally synthesized proteins actually contribute to synaptic modifications? The potentiation of synaptic transmission by neurotrophic factors in hippocampal slices displays a very early (~10 min) requirement for new protein synthesis (Kang and Schuman, 1996). Experiments in which the synaptic neuropil was isolated from the principal cell bodies indicate that the protein synthesis source is not somatic, strongly suggesting a dendritic origin (Kang and Schuman, 1996). Additional evidence comes from an enviable set of experiments in cultured *Aplysia* neurons (Martin et al., 1997). In these studies, the long-term synaptic facilitation induced by serotonin application was shown to rely on proteins synthesized locally in the axons of sensory neurons. More recently, Ouyang et al. (1997) showed that long-term potentiation (LTP) induction is accompanied by an increase in both phosphorylated and non-phosphorylated CAMKII detected in the dendrites of hippocampal slices. This increase occurred within 30 min of LTP induction, strongly suggesting that the kinase was synthesized in the dendrites. While the general requirement for CAMKII phosphorylation activity in LTP is clear, it is not yet known whether new, local synthesis of CAMKII is required for this activity. To address this general question of the necessity of dendritic protein synthesis for plasticity, techniques that permit the inhibition of dendritic mRNA availability or protein synthesis, exclusively, need to be developed.

When are newly synthesized proteins required during synaptic plasticity? As indicated above, neurotrophin-induced potentiation in hippocampal slices requires new protein synthesis within minutes (Kang and Schuman, 1996). Other forms of synaptic plasticity, including LTP in the hippocampus and long-term facilitation (LTF) in *Aplysia*, appear to exhibit a delayed requirement for new proteins (reviewed by Schuman, 1997). This notion is based on observations that LTP and LTF can be initiated and persist for at least an hour in the presence of translation inhibitors. This early protein synthesis–independent enhancement is followed by a slow decline to baseline levels, reflecting the requirement for new protein synthesis. From this, it is often inferred that somatically synthesized new proteins contribute to the later, enduring phases of synaptic potentiation. The locus (somatic or dendritic) of protein translation that gives rise to the protein synthesis dependence of LTP, however, has not been definitively established. The temporal delay in inhibition observed in the above experiments could very well reflect events that include signaling to the nucleus, gene transcription, the transport of mRNAs, and eventual translation at dendritic sites. The time requirements

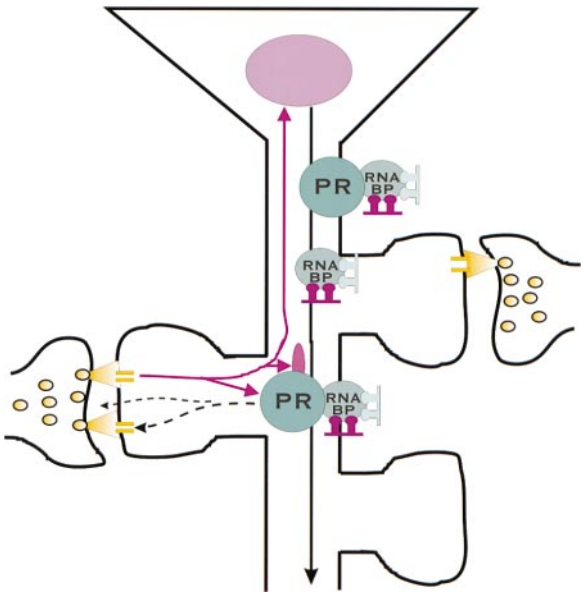

Figure 1. mRNA Trafficking and Local Protein Synthesis at the Synapse

mRNAs may be trafficked from the soma as complexes containing a heterogeneous complement of messages. These complexes may be associated with RNA-binding proteins and/or components of polyribosomes. Synaptic activity has three potential actions: (1) generation of signal(s) that travel to the nucleus to stimulate transcription and/or translation, (2) generation of a signal that serves to mark the synapse and recognize newly transported mRNA complexes, and (3) stimulation of protein translation at the synapse.

for these events, including the transport rates of mRNAs (~300 μm/hr), are consistent with this idea. Determining the locus of protein synthesis during plasticity will require techniques that allow the dynamic visualization of protein translation as well as methods for interfering exclusively with protein synthesis in dendrites.

If mRNAs are trafficked during synaptic plasticity, then one must consider again whether there is specificity in trafficking, such that some synaptic sites would receive the plasticity-induced mRNAs whereas others would not. The idea of a synaptic tag was recently advanced to explain how somatically synthesized proteins would be delivered to the appropriate sites during plasticity (Frey and Morris, 1997). In the context of mRNA delivery, there may exist a similar type of mechanism to capture the appropriate mRNAs during plasticity. Alternatively, the specificity may not be derived from availability of the message but rather from its usage. In this case, rather than employing selective mRNA trafficking, there would be selective site-specific translation of a globally distributed message. Does the transport of mRNAs, rather than proteins, offer any advantages to neurons when considering this problem of specificity? Arguments involving economy in biology are often dangerous, but one clear advantage is the relative numbers of mRNAs versus proteins that need to be trafficked into the dendrites. A single mRNA could, of course, provide the synapse with multiple copies of a given protein. In addition, it may be easier to ship mRNAs, since trafficked proteins could potentially be bound or sequestered at unwanted sites within the dendrite.

Conclusions

The local delivery of mRNA and synthesis of proteins in dendrites provides a solution to the problem of synaptic heterogeneity (Figure 1). Early work clearly demonstrating the presence of both mRNAs and polyribosomes in dendrites indicated the capacity for local translation. Recent studies have only begun to unravel the mechanisms by which mRNAs are trafficked to dendritic compartments and potential mechanisms for translational control. Both mRNA trafficking and local protein synthesis may contribute to synaptic changes that are restricted to particular dendritic regions. The development and application of molecular tools that permit a dynamic view of mRNA trafficking (e.g., Bertrand et al., 1998) and protein synthesis will undoubtedly advance our understanding of these processes.

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